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ANALYTICAL REFERENCE SERVICE TRAINING
PROGRAM. FRESHWATER PLANKTON, NUMBER 1,
NOVEMBER 1964

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio

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FRESHWATER PLANKTON NO. 1

Report of a study conducted by the
ANALYTICAL REFERENCE SERVICE
TRAINING PROGRAM

Public Health Service
Environmental Health
Reference Room

Department of Health, Education, and Welfare
Public Health Service
Bureau of State Services
Division of Water Supply and Pollution Control

Robert A. Taft Sanitary Engineering Center
Cincinnati, Ohio
1964

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This Study was Designed
And the Report Written

By

H. W. Jackson, Aquatic Biologist
And
M. E. Bender, Biologist

Water Supply and Pollution Control Training

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FOREWORD

The Analytical Reference Service is conducted by the Training Program of the Robert A. Taft Sanitary Engineering Center for evaluation of laboratory methods in the environmental field. Cooperative studies by member organizations, through analysis of identical samples and critical review of methodology, provide the mechanism for:

Evaluation of analytical procedures, including precision and accuracy, by comparison of the procedures and results reported by participating laboratories.

Exchange of information regarding method characteristics.

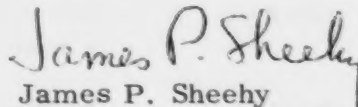
Improvement or replacement of existing methods by development of more accurate procedures, and development of new methodology for determination of new pollutional components.

Samples are designed to contain measured amounts of selected constituents. Decisions as to qualitative makeup are made by the ARS staff, the membership, and consultants. Notice of each study is sent to the entire membership.

A portion of the study sample is then shipped to all who express a desire to participate, with accompanying data forms for reporting numerical values, a critique of the procedures used, and comments on modifications, sources of error, difficulties encountered, or other pertinent factors. The results and comments of each study are compiled and a report is prepared.

Initially directed toward examination of water, studies now include air, milk, and food. Some studies are periodically repeated for the advantage of new members, to evaluate new methods, or to reevaluate existing methods.

The selection of studies is guided by the responses to questionnaires periodically circulated among the membership, which now includes 184 federal, state, and municipal agencies, industries, universities, consulting firms, and foreign agencies.


James P. Sheehy
Director, Training Program

STUDIES ON WHICH REPORTS HAVE BEEN COMPLETED

Water-Minerals	Calcium, magnesium, hardness, sulfate, chloride, alkalinity, nitrite, nitrate, sodium, and potassium. Studies completed in 1956, 1958, and 1961.
Water-Metals	Lead, copper, cadmium, aluminum, chromium, iron, manganese, and zinc. Studies completed in 1957 and 1962.
Water-Fluoride	Fluoride in the presence and absence of interferences, with and without distillation using a specified procedure. Studies completed in 1958 and 1961.
Water-Radioactivity	Studies completed in 1959, 1961, and 1963. The first two studies were designed to determine gross beta activity, while the third study was concerned with gross beta and strontium-90 activity.
Water-Surfactant	Surfactant in various waters. Studies completed in 1959 and 1963.
Water Oxygen-Demand	Biochemical oxygen demand and chemical oxygen demand. Study completed in 1960.
Water Trace-Elements	Arsenic, boron, selenium, and beryllium. Study completed in 1962.
Air-Inorganics	Chloride, sulfate, fluoride, and nitrate in aqueous solution and on glass fiber hi-vol filter mats. Study completed in 1958.
Air-Lead	Filter paper tape impregnated with lead. Study completed in 1961.
Air Sulfur-Dioxide	Determination of sulfur dioxide in air using a specified method. Study completed in 1963.
Milk DDT-Residue	DDT in milk. Study completed in 1962.

PARTICIPANTS IN THIS STUDY

Arkansas Water Pollution Control Commission
Baltimore Department of Public Works, Ashburton Filters
Benton Harbor Filtration Plant
California State Department of Health, Berkeley
California State Department of Health, Los Angeles
Cedar Rapids City Water Department
Chicago Department of Water and Sewers, South District Filtration Plant
Cincinnati Water Works
City of Evanston, Illinois, Water Department
City of Salem, Indiana, Water Department
City of Tacoma Water Division
Colorado State Department of Public Health
Connecticut State Department of Health
Denver Board of Water Commissioners
Department of Municipal Laboratories, Ontario
Environmental Health Laboratory, Manitoba
Flint Water Plant, Department of Public Works and Utilities
Gary-Hobart Water Company
Great Lakes - Illinois River Basins Project
Illinois State Department of Public Health, Chicago
Indianapolis Water Company
Instituto Ezequiel Dias-Divisao de Aguas, Brasil
Iowa City State Hygienic Laboratory
Long Beach Water Department
Los Angeles Department of Public Works, Hyperion Treatment Plant
Los Angeles Department of Water and Power
Louisiana State Board of Health
Louisiana Stream Control Commission
Massachusetts Department of Public Health
Metcalf and Eddy Laboratory, Boston
Metropolitan Water, Sewage, and Drainage Board, Sydney, Australia
Michigan State Department of Health
Milwaukee Water Works, Linwood Avenue Plant
New Jersey State Department of Health
New York State Conservation Department
North Carolina Department of Water Resources
Ontario Water Resources Commission
Philadelphia Suburban Water Company

Philadelphia Water Department (Belmont Laboratory)
Potamological Institute, Louisville
San Francisco Water Department
Santee Recreation Project Laboratory, California
St. Louis Water Division, Howard Bend Station
The Metropolitan Corporation of Greater Winnipeg, Waterworks Branch
The Water Research Association, Marlow, Buckinghamshire, England
Vermont State Department of Health
Washington State Department of Health

FRESHWATER PLANKTON NUMBER 1

OBJECTIVES OF THE STUDY

→ The increasing use of surface waters for municipal water supplies has increased the importance of plankton counting and enumerating in determining water quality. Attention accordingly is focused on the methods employed by laboratories which routinely count or otherwise analyze plankton, since the accuracy of any result depends to a large extent on the methodology employed, as well as on the training and skill of the analyst.

This study was conducted to determine the variations that may occur among the final results when many laboratories analyze aliquots of the same sample, and the extent to which the method of analysis affects the results obtained. Both qualitative and quantitative aspects were considered.

EVALUATION OF PRELIMINARY SAMPLE

Preliminary investigation and consideration led to the following conclusions:

1. A natural sample would be preferable to an artificial sample.
2. The Illinois River at Peoria, Illinois, contains a well-balanced selection of plankton types in the late fall and, at that time of the year, would provide a suitable source of water.

A pilot study was carried out to determine:

1. Whether the proposed preservative would stabilize the sample for an adequate period.
2. Whether the dispensing procedure would yield twenty 1-liter aliquots with virtually identical plankton counts.

During the pilot study, the twenty aliquots were analyzed on a rotating schedule for 65 days. Statistical analyses were run on errors arising from the sample subdivision, the effect of storage time, aliquot size, and the variance between individual aliquot counts and multiple counts on a single aliquot. The "t" test and analysis of variance were employed. No significant differences were detected at the 95% confidence level. Samples counted six months after collection still showed no change.

REFEREES AND REFEREE ANALYSES

The Referees

Serving as referees for the study were three groups of biologists, hereafter referred to as referee groups, or, individually, as analysts. They included:

Dr. H. W. Jackson and Michael E. Bender, Water Supply and Pollution Control Training, Training Program

Drs. C. M. Palmer and Louis G. Williams, Aquatic Biology Section, Basic and Applied Sciences, Division of Water Supply and Pollution Control

Mrs. Julia M. Maloney, Mrs. Maryjo Sage, and Messrs. Eugene W. Hansmann and Louis E. Grivetti, National Water Quality Network,* Division of Water Supply and Pollution Control.

Aliquots were chosen at random for referee analysis. One aliquot was sent to Referee-Group II, one to Referee-Group III, and 4 were retained by Referee-Group I.

Technique Employed by Referees

Before distributing the aliquots, participating laboratories were requested to supply information on the procedures which they normally employ (Appendix 1). On the basis of this information, it was decided that the referee groups would evaluate the samples by means of a direct clump count of one strip in a Sedgwick-Rafter cell at 200 X magnification (See Appendix 1 for definition of terms. For detailed explanation of methods consult reference 5).

*Now known as the Water Pollution Surveillance System, DWS&PC.

Referee Results

Thirty counts made by the referee groups during an 8-week period resulted in a mean count of 23,500 per ml, with a standard deviation of 1,085 as shown in Table 1. Twenty of these counts were made by Referee Group I, from among the 4 aliquots selected at the time of sample division. The remaining ten counts were made by Referee Groups II and III.

PREPARATION OF ALIQUOTS FOR DISTRIBUTION TO THE LABORATORIES

Thimerosal stabilizer¹ was placed in each of twelve 5-gallon polyethylene carboys in an amount to make a 3 1/2% solution when filled. The carboys were sent to the Water Plant at Peoria, Illinois, where, on April 9, 1963, they were filled with raw river water. They were well-shaken to insure thorough mixing and were returned to Cincinnati. The day after they were received at the ARS laboratory (April 10), all carboys were reshaken and the contents poured into a large polyethylene tank from which aliquots were drawn off into polyethylene shipping containers for distribution to the laboratories. Preceding and during the dispensing procedure, the sample was stirred vigorously by a hand paddle. This method of stirring was chosen in preference to mechanical stirring to avoid setting up a swirl or flow pattern which might lead to differences among the aliquots as the water level in the tank dropped.

RESULTS FROM PARTICIPANTS

Analysis of Identification Data

One hundred eleven genera were reported by 44 laboratories. Seven genera were judged by the referees to be invalid, leaving 104 genera on which the following remarks are based (Tables 3 and 8). The validity of any given genus was established on the basis of the professional opinion of three individual referees who were selected to make final decisions on taxonomy. They considered the likelihood of the occurrence of that particular genus in the Illinois River in the late fall, based on personal experience, information reported in the literature, or both. When they agreed that the probability for some particular genus was virtually zero, it was ruled that, for the purposes of this study, a report of this genus was a taxonomic error. The 7 invalid genera are included, and so indicated, in Table 2, but nowhere else.

When one or two of the three referees felt that a given genus might have been present (even though a remote possibility), that genus was listed as "unlikely." Eleven genera came under this category, though no more

than three laboratories reported any one of these genera. They are listed in Table 3-B, but were not excluded from consideration or calculation at appropriate points in the study.

Ten genera reported by the referee groups were not reported by any of the participating laboratories, while 26 possibly valid genera were reported by only one laboratory (Table 8). That the laboratories contributed a higher number of genera than was reported by the referee groups was to be expected, since the probability of encountering and identifying the total number of forms that may have been present favored the group which analyzed the larger portion of the sample (the 44 participating laboratories running triplicate analyses). The largest number of genera reported by any analyst was 34. The mean of all analysts was 17. The referees reported 53 genera in all (Tables 2 and 8). All genera reported are listed in Table 2. They are grouped as blue greens, flagellates, nonmotile greens, diatoms, and miscellaneous. No corrections for misidentifications were applied to the total counts, as presumably these were not greatly affected by identification errors.

Majority rule does not, per se, validate taxonomic determination, but, nevertheless, it is interesting that the genera reported fall into two distinct groups (Figure 1):

1. Genera (13) reported by more than half of the laboratories
2. Genera (91) reported by less than half of the laboratories

All genera in the first group were adjudged to have been actually present, as all were substantiated by the referee laboratories (Table 2). This group constitutes the bulk of the total count, although a few high counts appear for other less frequently reported genera, such as Chlorella, No. 5; Anacystis, No. 55; and Diatoma, No. 94. The genera comprising the second group were reported by 17 laboratories. The separation of reported genera into the two groups is important for the following reasons:

The nature of each of the 13 widely reported genera suggests that certain characteristics contributed to their recognition: they were large and conspicuous like Euglena, No. 69 or Scenedesmus, No. 1; they were distinctive in appearance like Pediastrum, No. 3 or Asterionella, No. 85; or they were abundant like Cyclotella, No. 88 or Stephanodiscus, No. 90. In general it may be assumed that opposite characteristics were possessed by the remaining 91 genera. It is also likely that many of this group were falsely identified (Table 3).

Occasional misidentification is inevitable with a group of 44 analysts examining unfamiliar material, and with thousands of species and genera to choose from in the literature. Unfortunately we cannot know exactly which listings are in error, but each mistake is potentially important. Error must be recognized as a factor contributing to the large number of genera reported by but one or a few laboratories. The wide range in counts reported for the genera in Table 2 may result in part from a lack of consistency in identification. Even for the genera classified as potentially valid, identification ranged from the laboratories which did not detect or perhaps recognize a genus at all, through those which apparently recognized one or a few, to those who counted many (Table 4). This lack of consistency may be responsible for the fact that the means reported in Table 2 tend to bear little resemblance to the individual differential counts reported by the laboratories. This evidence of taxonomic error suggests that error also can be a factor in routine plankton examination.

Another factor which contributed to the long lists of seldom-reported genera may have been the actual, rare occurrence of many different forms. In a rich and varied natural sample, it is impossible to certify that one or more specimens of a particular form may or may not have been present in one given aliquot and absent from others. That only one or two laboratories recognized a particular genus does not necessarily signify that that genus was misidentified. The genus may have been a rare form which did not appear in all samples.

Ecologists recognize that while a few relatively common forms appear frequently in a community, less common forms also appear. The number of less common and rare forms has been shown to be greater than the number of common forms.² It is therefore entirely reasonable that the numbers of different genera found by but one or a few laboratories should be large. A little arithmetic shows that the 12 carboys of the original sample, holding approximately 20 liters each, contained enough water to fill well over 2 billion Whipple counting fields at 100X magnification. It was necessary for only one lone algal cell to be counted once in one single Whipple field by one analyst to be listed among the rare genera.

Analysis of Counting Data

Since the laboratories participating in the study were instructed to employ whatever procedures they normally use, the analyses were reported in a variety of ways. Table 5 shows the combinations of techniques which were most frequently employed, and some of the final results. In all, the direct count procedure was used by 22 laboratories, the traditional Sedgwick-Rafter procedure by 15, the

Plankton Centrifuge by 5, and the Membrane Filter by 3. Two other procedures were reported by individual laboratories, one of which employed a modification of the Koffoid method, the other a unique combination of procedures. The number of laboratories which used the various procedures does not always balance in the remaining sections of this report for the following reasons:

1. Some laboratories reported results obtained by two or more methods.
2. The statistical analyses shown in Table 5 were prepared from the group of reports selected according to the criteria explained in Treatment of Data, below.
3. One laboratory reported only final results in terms of areal standard units without including intermediate counts.

Treatment of Data

The counts were plotted on normal probability paper (See log probability curve, Appendix 2), and 12 were judged not to be part of the normal distribution for purposes of this study. These outlying values are circled on the probability curve and are not included in Table 5.³ It should be emphasized that elimination of a participant's results in this manner does not in itself imply that the results were invalid, but only that they were not a part of a normal distribution within which valid statistical comparisons can be made. Likewise, combinations were not included. Laboratories which utilized more than one magnification in making their analyses are included in the group making counts at 200X. Certain workers counted large forms at low magnification and small forms at high magnification.

Discussion of Techniques

Counts in Table 5 are separated into clump-type versus individual-cell counts (See Appendix 1), but, in Figures 2 and 3, all counts are recorded as reported without reference to type or to their inclusion in Table 5. This procedure was considered useful in this case, since very little clumping occurred in the sample, and the two groups of counts were very close together. No referee value was determined in areal or cubic standard units since this method is not normally employed at SEC. In all cases but one, however, those making final reports in standard units also reported preliminary intermediate counts which could be used in this report. Standard unit results are given in Table 7.

Significance of Magnification

The one aspect of technique which most consistently affected the final count was magnification. Those using higher magnification obtained results closer to the referee count than those using lower magnification. Bar graphs (Figures 2 and 3) of those counts that could be approximated as 100X or 200X (or greater) magnification, show their relationships to the referee count (23,500 per ml). All numerical counts of whatever type are included here, in contrast to the numerical analysis in Table 5 (Appendix 2). Note that in Figure 2 all but two of those counting at 100X obtained final counts well below the referee score. Those counting at 200X and higher magnifications (Figure 3) obtained results which were well distributed on both sides of the mean, indicating that variations here were due to factors other than magnification. Referee analysts generally preceded their official count with a qualitative study using oil immersion or at least 400X magnification to determine the exact types of plankton actually present, before beginning official quantitative counts.

Effect of Preparation Technique

Due to the small number of laboratories involved, statistical inferences are not warranted as to the relative merits of the three most frequently employed techniques for preparing microscopic mounts. Inspection of Figures 2 and 3, however, reveal interesting relationships.

The Sedgwick-Rafter filtration procedure was employed by 15 laboratories, 7 using 100 X magnification and 8 using 200 X. Note that at 100 X, not one laboratory obtained a count as high as the referee value, and 5 of the 7 were less than 1,000 per ml. Most of the nanoplankton apparently were lost by this procedure, except for the two counts shown in Figure 2 (made at 200 X magnification), which were considerably above the referee count. These were, however, the only high counts in the entire group of 15 Sedgwick-Rafter filtrations. Further, the numerical mean of the 9 that could be analyzed (Table 5) was also low (13,204 per ml). Laboratories using this technique obviously lost a large portion of the organisms.

Much the same type of situation seems to exist for the plankton centrifuge, although only 5 laboratories were represented in this group. An important difference, however, is the absence of extremely low counts in this group. The mean, as shown in Table 5 (14,729 per ml), is slightly closer to the referee count of 23,500.

The direct-count method was the most accurate, with several of the results approaching the referee value very closely. Nearly all of the higher counts were made by this procedure. Over half of the direct counts were above the referee value, as shown in Figure 3. The mean of the statistically acceptable group in Table 5 (23,870) is almost exactly that of the referee count. In spite of occasional extreme variation, the direct-count procedure appears to be definitely superior; it obviously has the added operational value of simplicity.

The few participants who employed techniques not used by others are included as miscellaneous in Figures 2 and 3, and may be identified by laboratory number in Tables 6 and 7.

DISCUSSION

Of the ten genera reported by the referees only (Table 8), none was present in large numbers, and the same was true of those reported by the laboratories only. Of the rare forms reported by one to three laboratories, regardless of whether or not they were reported by the referees, few were present in more than a very few hundred per ml, and usually in numbers of 20 to 50 or 60.* This relationship would be expected of a group of forms which are actually rare.

Most laboratories which detected minute forms, such as Cyclotella, No. 88 and Stephanodiscus, No. 90, also recognized their great abundance; those which recognized such distinctive forms as Ankistrodesmus, No. 2, Pediastrum, No. 3, Euglena, No. 69, Asterionella, No. 85, Synedra, No. 87, Tabellaria, No. 91, and Fragillaria, No. 92 were also in most cases able to report their relative scarcity. Scenedesmus, No. 1 was almost universally recognized as present in modest numbers.

The 93 genera less often found represent an ever present problem in routine plankton-counting operations. For any given body of water, there will be a group of commonly reported genera (Table 4). From time to time, additional kinds will be reported. When a new kind appears in a record, it is important to ascertain whether it is a rare type newly reported, or a type which may have been present but never before recognized. If the latter, it is conceivable that unexplained conditions in the past may have been due to this organism. Any natural body of water tends to have many forms of life; some are easily found, others not. This study demonstrates the principle that continuing study of a given sample tends to reveal more and more types. It is estimated, on the basis of procedures proposed by Preston,² that if study of this particular sample could be continued long enough, as many as 250 genera might eventually be identified.

At first glance, this report might give the impression that plankton counting is a futile exercise. Basically, the objectives were concerned with an inventory of laboratory procedures and performance, not with establishment of the relative rank or proficiency of the participating laboratories. A sample was chosen for analysis that contained forms unfamiliar to many of the analysts. In one respect this might be regarded as a handicap; on the other hand, it provided a practical test of the

*In most cases the reported count probably represented the multiplication of a single observed plankter by the factor used to convert raw counts to counts per ml. Many analysts, on the other hand, simply reported single observations for a count of 1 per ml.

capacity of a group of operating laboratories to find and identify plankton. Many of the participants are concerned in their routine work with the plankton of only a limited region, or even of a single body of water. Only three replicate sets of observations or counts were specified in the study (Appendix 1). As mentioned above, as many as 250 genera might have been listed. Hence, no single analyst could have been expected to find more than a portion of those present. The seven "expert" referee analysts found only 40% of the total number reported. A considerable range of counts and variety of identifications were therefore anticipated. Gamet and Rademacher,⁴ in their study of water works operators in the Great Lakes region, likewise encountered great variation.

Magnification is shown to be a factor of prime importance in the detection and recognition of minute forms. It is fortunate that most microscope manufacturers now offer an objective lens with a magnification of approximately 20 X or 21 X which can be used with a Sedgwick-Rafter cell. Since the magnification of a microscope is the product of magnification of the objective multiplied by the magnification of the eyepiece, the total magnification of a scope fitted with a 10 X eyepiece and a 10 X objective is 10×10 or 100. The addition of a 20 X or 21 X objective thus approximately doubles the magnification formerly available, and adds greatly to the analyst's ability to enumerate the smaller plankton organisms.

Not all laboratories routinely attempt to count all plankton organisms present. Some search only for particular forms known to be trouble-causers, others count only larger forms, such as microcrustacea. It would be unreasonable to expect an analyst accustomed to such procedures to locate and identify the entire nannoplankton flora of this sample, without special training. It might be advisable to consider whether or not nannoplankton may at times be responsible for effects on water supplies not attributable to larger forms.

SUMMARY

This study has revealed a wide diversity among results reported by the participating laboratories, both in the genera of organisms recognized and in the total counts. Factors influencing the observed diversity appear to include relative skills of analysts, methods of preparing samples for microscopic examination, magnification used, and random variations in the contents of the Whipple fields actually examined.

The study demonstrates a need for improvement and standardization of techniques for plankton analysis. Training to improve analytical skills and standardization of techniques can lead to more useful and meaningful evaluation of a given water and to the exchange of information among laboratory personnel working on similar problems.

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5. Standard Methods for the Examination of Water and Waste Water. 11th Edition. APHA.

Table 1. REFEREE DIFFERENTIAL COUNTS PER ML

Groups	Diatoms	Nonmotile green algae	Flagellates	Blue-green algae
Mean	22,880	1,180	390	80
Minimum	20,298	563	205	0
Maximum	24,576	1,887	666	256

Mean total count, 23,500

Standard deviation, + 1,085

Number of counts, 30

Table 2. GENERA REPORTED¹

Genus	Genus No.	No. of laboratories reporting this genus	Mean count per ml.	Minimum	Maximum
<u>NONMOTILE GREENS</u>					
Scenedesmus*	1	44	724	1	2100
Ankistrodesmus*	2	29	387	1	1764
Pediastrum*	3	25	104	1	1886
Micratinium*	4	24	786	1	5200
Chorella*	5	16	1794	5	13693
Closterium*	6	16	101	1	745
Coelostrum*	7	9	68	7	167
Ulothrix	8	8	28	3	54
Selenastrum*	9	7	120	1	565
Zygnema	10	6	27	1	74
Dictyosphaerium	11	5	194	9	600
Microspora	12	5	44	1	102
Mougeotia*	13	5	72	2	139
Actinastrum*	14	4	78	4	282
Chlorococcum	15	4	25	2	40
Crucigenia*	16	4	77	1	141
Phytoconis**	17	4	170	73	354
Spirogyra	18	4	11	1	28
Closteriopsis	19	3	27	7	40
Cosmarium*	20	3	2	1	3
Oocystis*	21	3	90	40	140
Schroederia	22	3	167	1	333
Tetraspora	23	3	68	33	113
Chodatella	24	2	11	1	20
(Lagerheimia)*					
Desmidium	25	2	37	7	61
Gleocystis	26	2	113	32	194
Palmella	27	2	270	8	533
Protococcus**	28	2	2	1	2
Sphaerocystis*	29	2	39	---	
Hormidium	30	2	103	48	159
Tetraedon*	31	2	11	2	20
Roya	32	2	3	---	
Botryococcus**	33	1	35		
Centritractus	34	1	10		
Golenkina*	35	1	308		

1. Listed in descending order of number of laboratories reporting each genus.

* Found by referees.

** Invalid (Table 3)

Genus	Genus No.	No. of laboratories reporting this genus	Mean count per ml.	Minimum	Maximum
Kirchneriella*	36	1	1		
Ourococcus	37	1	38		
Sphaeroplea**	38	1	40		
Eremosphaera**	39	1	8		
Treubaria	40	1	61		
Franceia	41	1	T		
Nephrocytium	42	1	20		
Palmellococcus	43	1	20		
Spirotaenia	44	1	T		
Staurastrum*	45	1	T		
Tribonema	46	1	5		
Xanthidium	47	1	1		
Zoochlorella	48	1	300		
Dimorphococcus*	49	0			
Nannochloris*	50	0			
Pachycladon*	51	0			
Elaktothrix*	52	0			
Tetrastrum*	53	0			
Tetradesmus*	54	0			

BLUE GREENS

Anacystis*	55	10	579	3	4533
Oscillatoria*	56	9	26	1	88
Agmenellum*	57	6	123	7	533
Coelosphaerium	58	4	320	2	805
Chroococcus*	59	4	19	1	60
Spirulina	60	4	22	1	40
Anabaena	61	3	7	1	15
Gomphosphaeria	62	2	15	7	22
Aphanizomenon	63	1	3		
Lyngbya	64	1	135		
Nostoc**	65	1	2		
Phormidium*	66	0			
Raphidiopsis*	67	0			
Coccochloris*	68	0			

Genus	Genus No.	No. of laboratories reporting this genus	Mean count per ml.	Minimum	Maximum
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FLAGELLATES

Euglena*	69	29	245	1	1452
Trachelomonas*	70	11	45	1	173
Phacus*	71	7	136	3	533
Chlamydomonas*	72	3	186	28	480
Pandorina*	73	2	11	1	20
Peridinium	74	2	10	---	
Chrysococcus*	75	1	7		
Dinobryon	76	1	55		
Eudorina	77	1	1		
Pleodorina	78	1	1		
Volvox	79	1	10		
Uroglenopsis	80	1	20		
Chromulina*	81	0			

MISCELLANEOUS

Rotifer	82	1			
Vorticella	83	2	53	28	78
Monad*	84	1	20		

DIATOMS

Asterionella*	85	43	193	4	989
Navicula*	86	40	460	1	9600
Synedra*	87	37	260	2	1568
Cyclotella*	88	35	8937	26	23386
Melosira*	89	34	808	9	12063
Stephanodiscus*	90	31	3181	2	26333
Tabellaria*	91	31	118	1	2375
Fragilaria*	92	24	124	1	736
Surirella*	93	17	122	6	347
Diatoma*	94	16	785	3	9267
Nitzschia*	95	13	187	1	1320
Gomphonema*	96	9	103	1	506
Cymbella	97	8	20	1	100
Stauroneis	98	6	13	2	44
Pinnularia	99	5	12	1	32
Gyrosigma	100	4	118	3	294

Genus	Genus No.	No. of laboratories reporting this genus	Mean count per ml.	Minimum	Maximum
Meridon	101	4	23	10	32
Pleurosigma	102	4	53	5	101
Rhizosolenia	103	3	52	1	103
Amphora	104	2	29	1	57
Coscinodiscus*	105	2	54	9	100
Neidium	106	2	11	1	20
Cymatopleura	107	2	12	5	20
Achnanthes	108	1	9		
Attheya**	109	1	17		
Cocconeis	110	1	1		
Epithemia	111	1	1		

Table 3. FALSE AND UNLIKELY IDENTIFICATIONS

A

Identifications considered by three or more of the referees to be definitely in error (Genus numbers refer to Table 2).

Phytoconis, No. 17 and Protococcus, No. 28
(Terrestrial genera. Forms observed
may have been clumps of Chlorella.)

Botryococcus, No. 33 (Reported as sporadic
in occurrence, not a river form.)

Sphaeroplea, No. 38 (Reported as sporadic
in occurrence, not a river form.)

Eremosphaera, No. 39 (Large, distinctive; a
soft water form.)

Nostoc, No. 65 (Colonies rarely planktonic,
filaments resemble Anabaena.)

Attheya, No. 109 (Essentially marine. Reported
for Ohio River, but not from Illinois River.)

B

Genera considered to be unlikely by one or two referees. One or more specimens might possibly have been included in the original 60-gallon sample. No more than three laboratories reported any one genus.

Genus	No. of genera
<u>Closteriopsis</u>	19
<u>Roya</u>	32
<u>Centritractus</u>	34
<u>Ourococcus</u>	37
<u>Treubaria</u>	40
<u>Nephrocytium</u>	42
<u>Spirotaenia</u>	44
<u>Zoochlorella</u>	48
<u>Pleodorina</u>	78
<u>Volvox</u>	79
<u>Uroglenopsis</u>	80

Table 4. MOST REPORTED GENERA

Genus	Genus No.	No. of laboratories reporting this genus	Mean count per ml.	Minimum	Maximum
Scenedesmus	1	44	724	1	2100
Asterionella	85	43	193	4	989
Navicula	86	40	460	1	9600
Synedra	87	37	260	2	1568
Cyclotella	88	35	8937	26	23386
Melosira	89	34	808	9	12063
Stephanodiscus	90	31	3181	2	26333
Tabellaria	91	31	118	1	2375
Ankistrodesmus	2	29	387	1	1764
Euglena	69	29	245	1	1452
Pediastrum	3	25	104	1	1886
Micratinium	4	24	786	1	5200
Fragilaria	92	24	124	1	736

Table 5. ANALYTICAL PROCEDURES EMPLOYED*†

Direct counts				Sedgwick-Rafter Filtration		Plankton Centrifuge		Membrane Filter
200 X		100 X		200 X		200 X	100 X	All magnifications
Individual	Clump	Individual	Clump	Individual	Clump	Individual	Individual	Individual
4 L $\bar{X}$ 27,708	5 L $\bar{X}$ 20,032	5 L $\bar{X}$ 10,724	4 L $\bar{X}$ 15,971	2 L $\bar{X}$ 26,774	5 L $\bar{X}$ 8,419	3 L $\bar{X}$ 19,669	2 L $\bar{X}$ 7,310	1 L $\bar{X}$ 1,809
9 L $\bar{X}$ 23,870	9 L $\bar{X}$ 13,056	7 L $\bar{X}$ 12,737	2 L $\bar{X}$ 14,523	5 L $\bar{X}$ 14,729	1 L $\bar{X}$ 1,809			
18 L $\bar{X}$ 18,145		9 L $\bar{X}$ 13,204		5 L $\bar{X}$ 14,729				

*Mean referee count, 23,500 (Table 1)

†Some laboratories using miscellaneous methods are not included in this table.

LEGEND: L = Laboratories employing this procedure. One to three separate counts were reported by each laboratory.

 $\bar{X}$ = mean

Table 6. DATA REPORTED IN COUNTS

Laboratory No.	Replicate counts	$\bar{X}$	Method of concentration	Magnification	Type of count
01	35,380 32,700 31,720	33,265	Direct	200	Individual
02	7,199 7,022 7,593	7,270	Plankton Centrifuge	100	Individual
03	32,737 19,864 22,100	24,900	Direct	210	Individual
07	16,110 14,900 26,080	19,031	Direct	100	Clump
010	3,700 4,520 4,400	4,207	Direct	100	Clump
011	29,050 30,170 27,790	29,003	Direct	100	Clump
012	39,800 42,800 42,300	41,601	Direct	100	Clump
014 A	25,692	25,692	Sedgwick-Rafter	200	Individual
014 B	13,249	13,249	Sedgwick-Rafter	200	Clump
015	7,390 7,100 7,610	7,370	Plankton Centrifuge	100	Individual
016	25,600 20,800 28,800	25,067	Direct	200	Individual
017	14,625 19,215 14,511	16,117	Direct	200	Clump

Laboratory No.	Replicate counts	$\bar{X}$	Method of concentration	Magnification	Type of count
019	11,189 11,473 12,273	11,643	Direct	100	Clump
020	113 150 333	199	Direct	100	Clump
021	146 138 122	136	Sedgwick-Rafter	80	Clump
022	24,955 28,286 28,163	27,134	Sedgwick-Rafter	200	Individual
023	23,695 27,759 26,923	26,127	Direct	200	Clump
025	488 504 526	504	Membrane Filter	200	Clump
027	393 498 510	467	Sedgwick-Rafter	100	Clump
028	221 300 264	262	Sedgwick-Rafter	100	Individual
029	154,823 177,293 227,772	186,629	Direct	200	Individual
030	28,100 26,000 28,700	27,599	Direct	200	Individual
031	1,130 1,900 1,890	1,640	Direct	125	Individual
1211	3,499 3,573 3,735	3,603	Sedgwick-Rafter	210	Clump

Laboratory No.	Replicate counts	$\bar{X}$	Method of concentration	Magnification	Type of count
1311(a)	15,288 15,135 15,210	15,211	Sedgwick-Rafter	200	Clump
1311(b)	1,777 1,757 2,123	1,886	Sedgwick-Rafter	200	Clump
1611	796 636 542	658	Sedgwick-Rafter	100	Individual
1811	14,362 13,026 11,857	13,082	Direct	200	Clump
2711	25,795 25,795 26,467	26,019	Plankton Centrifuge	430	Clump
3111	1,646 1,944 1,839	1,809	Membrane Filter	High power	Individual
3411	10,430	10,430	Direct	210	Clump
3731	720 680 705	720	Sedgwick-Rafter	100	Individual
4421	21,954 17,938 17,314	19,069	Plankton Centrifuge	200	Clump
4511	19,330 19,450 25,470	21,416	Direct	100	Individual
4523	31,328 32,648	31,988	Direct	200	Clump
4711	87 87 81	85	Sedgwick-Rafter	100	Clump
6111	851 812 894	847	Membrane	837	Individual

Laboratory No.	Replicate counts	$\bar{X}$	Method of concentration	Magnification	Type of count
6311	12,701 13,001 13,752	13,149	Sedgwick-Rafter	100	Individual
6314	6,672 9,240 8,368	8,095	Direct	100	Individual
6424	52,700 56,100 57,400	55,400	Direct	480	Individual
6812	17,880 13,808 16,000	15,896	Sedgwick-Rafter	100	Individual
7112	2,436 13,263 17,620	11,107	Kofoid	100	
7222	35,035 30,813 23,094	29,647	*	250	Individual
7722	10,251 11,357 11,828	11,146	Direct	83	Individual
7912	11,690 13,200 9,080	11,323	Direct	100	Individual
8222	14,459 13,690 13,613	13,921	Plankton Centrifuge	322	Clump
8812	11,600 9,280 13,220	11,899	Sedgwick-Rafter	100 200 400	Clump

*Direct and Centrifuge

Table 7. DATA REPORTED IN STANDARD UNITS

Laboratory No.	Replicate unit values	$\bar{X}$	Method of concentration	Magnification	Type of count
Areal Standard Units					
01	12,900 8,400 7,300	9,605	Direct	200	Individual
013	24,332 22,638 20,405	22,458	Sedgwick-Rafter	200	Individual
021	170 222 136	176	Sedgwick-Rafter	80	Clump
022	6,917 8,976 7,894	7,929	Sedgwick-Rafter	200	Individual
028	547 628 568	581	Sedgwick-Rafter	100	Individual
029	18,465 13,082 15,424	15,657	Direct	200	Individual
030	8,400 7,980 8,190	8,187	Direct	200	Individual
1314	906 612 734	751	Direct	200	Individual
1611	231.4 93.8 81.4	109.2	Sedgwick-Rafter	100	Individual
3731	349 302 356	336	Sedgwick-Rafter	100	Individual
7112	1,799 7,275 2,925	4,000	Kofoid	100	

Laboratory No.	Replicate unit values	$\bar{X}$	Method of concentration	Magnification	Type of count
8812	2,344	2,516	Sedgwick-Rafter	100	Clump
	1,612			200	
	1,978			400	
Cubic Standard Units					
03	2,900	2,309	Direct	210	Individual
	2,030				
	1,997				

Table 8. MISCELLANEOUS STATISTICS

Number of laboratories participating	47
Number of laboratories reporting differential counts	44
Largest number of genera reported by any laboratory	33
Mean per laboratory	17
Maximum number of valid genera reported by a single laboratory	33
Total genera reported by referees	53
Genera reported by only one laboratory	26
 Total genera reported	 111
Genera judged invalid (Table 3)	<u>7</u>
Possibly valid genera remaining	104
 Genera more frequently reported (Table 4)	 13
Genera less frequently reported	<u>91</u>
Total genera	104
 Genera reported by referees only	 10
Genera reported by laboratories only	51
Genera reported by both laboratories and referees	<u>43</u>
Total genera reported	104

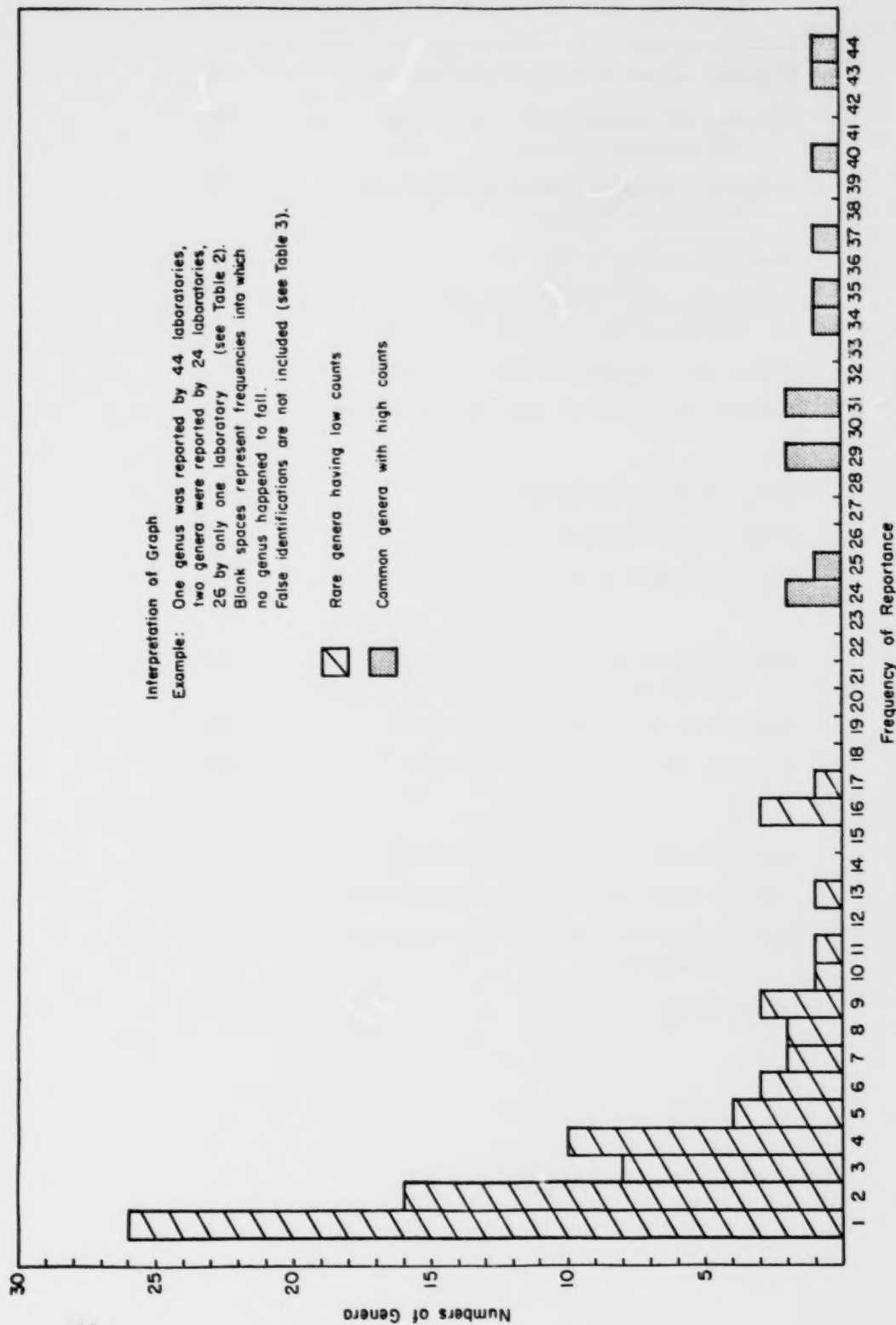
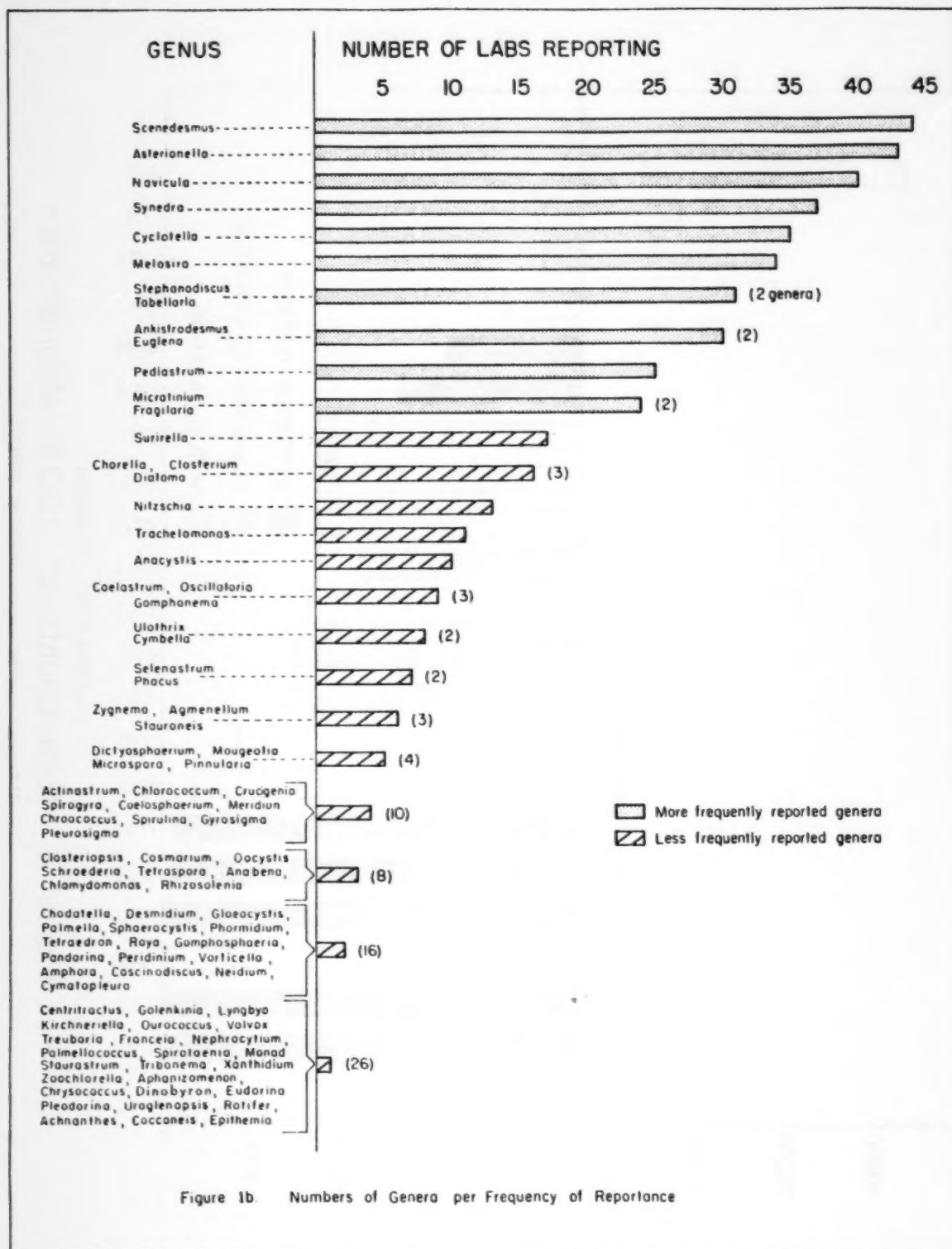


Figure 1a. Numbers of Genera per Frequency of Reportance



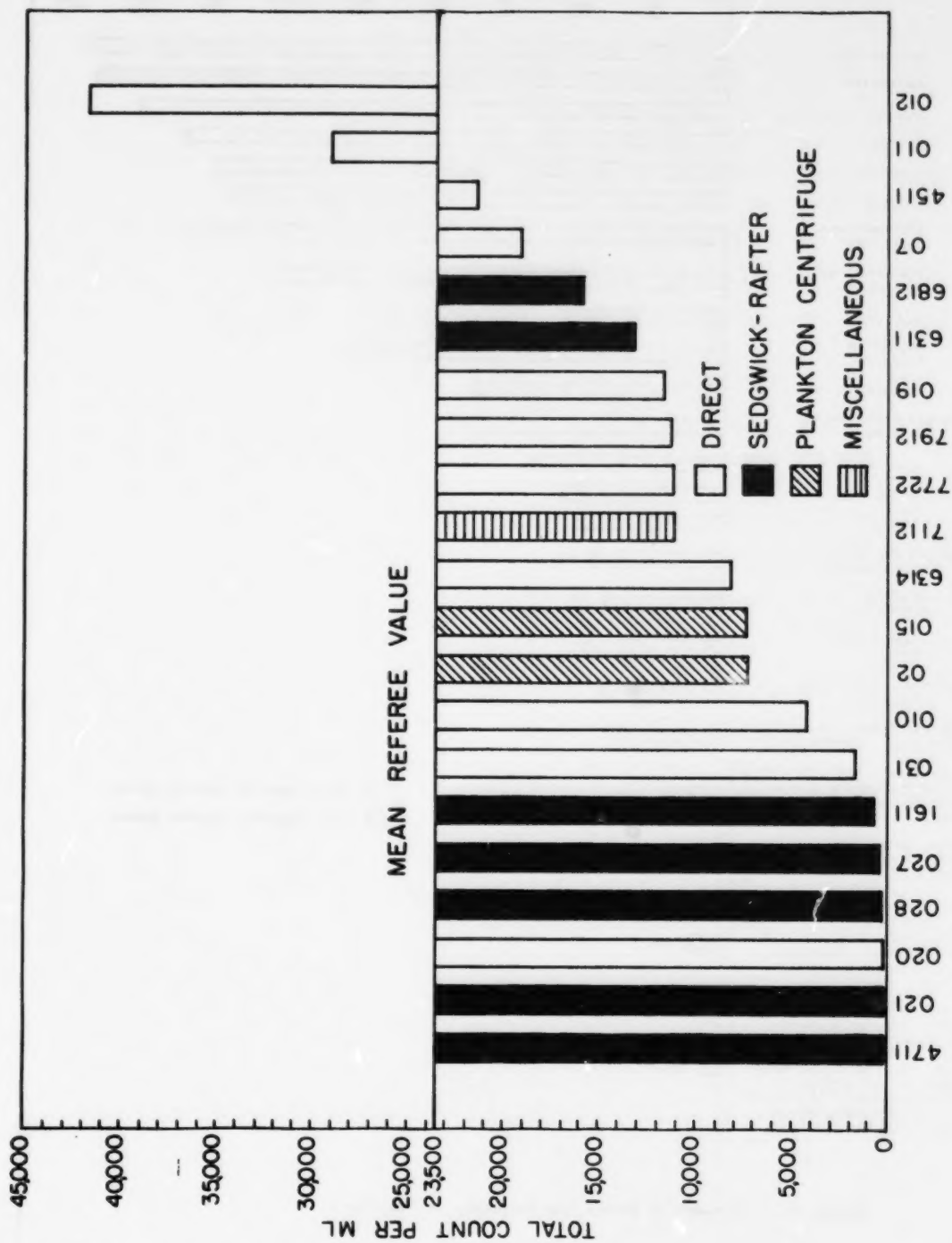


Figure 2. Total Counts at 100X Magnification
(Referee counts were made at 200 x)

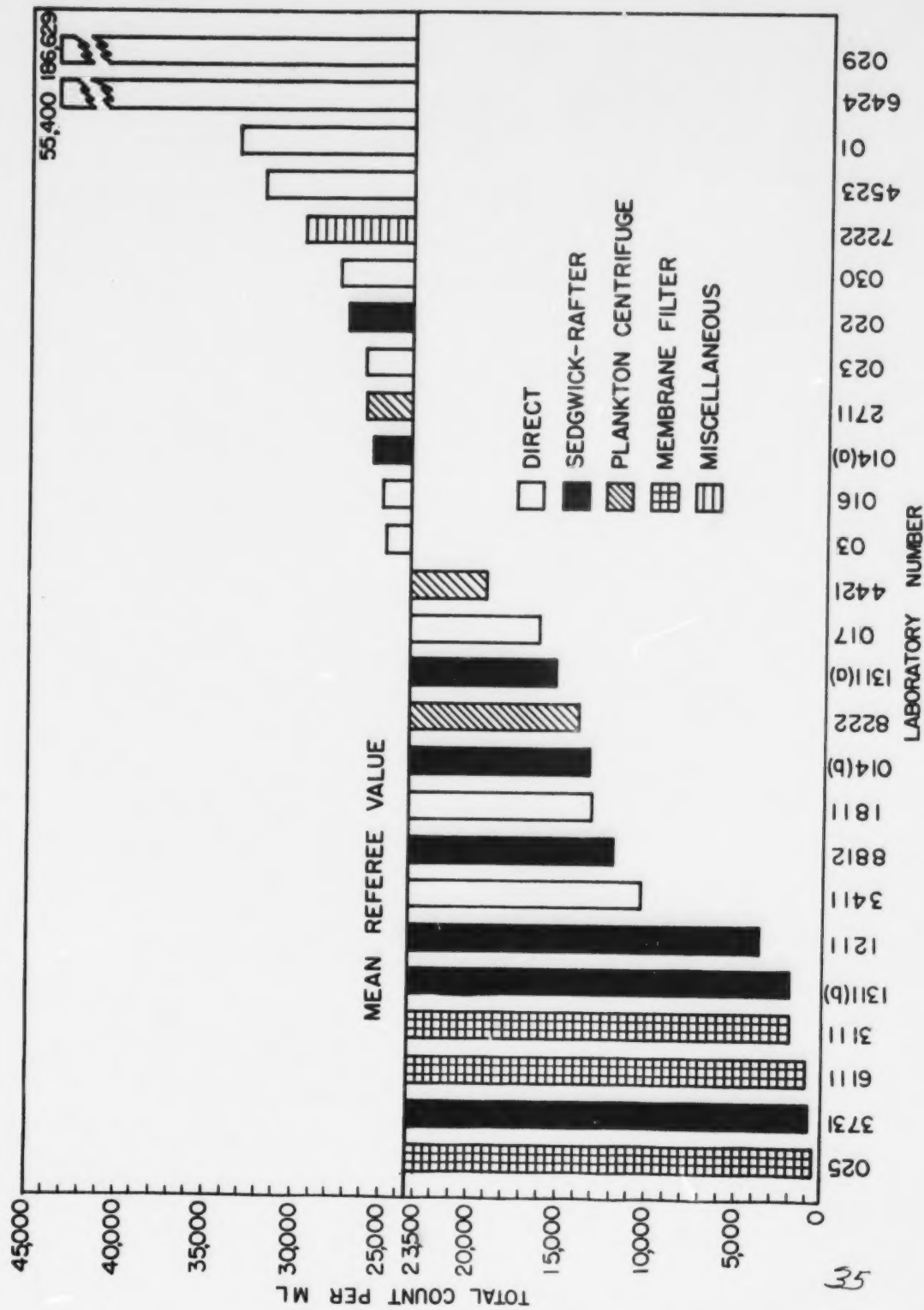


Figure 3. Total Counts at 200X Magnification

(Referee counts were made at 200 x)

APPENDICES

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Analytical Reference Service
FRESHWATER PLANKTON NO. 1
Information Sheet

Analysis of the count data from this study is dependent upon exact knowledge of the counting and preparation methods you employ. We have prepared specifications of the methods as we define them. If your methodology varies from the outlined methods, please state the differences in detail.

You are requested to perform all analyses in triplicate and report on the attached data forms. An additional set of forms is included for your own files. The sample should be stored in the dark or left in the closed cardboard carton. Since the sample is preserved, refrigeration is not necessary.

DEFINITION OF METHODS OF CELL COUNTING:

Clump Counts: In this method, each cell or clump of cells is counted as one. Filaments, colonies, or other such associations count no more than isolated separate cells.

Individual Cell Counts: In this method each cell is counted individually. When the cells occur in clumps or filaments, each of the cells in the clump or filament is enumerated.

Areal Standard Units: When employing this method, the size of the cells is taken into consideration. However, the number of cells of each type must be recorded in the preliminary analysis. Please report preliminary cell counts as well as final areal standard unit values.

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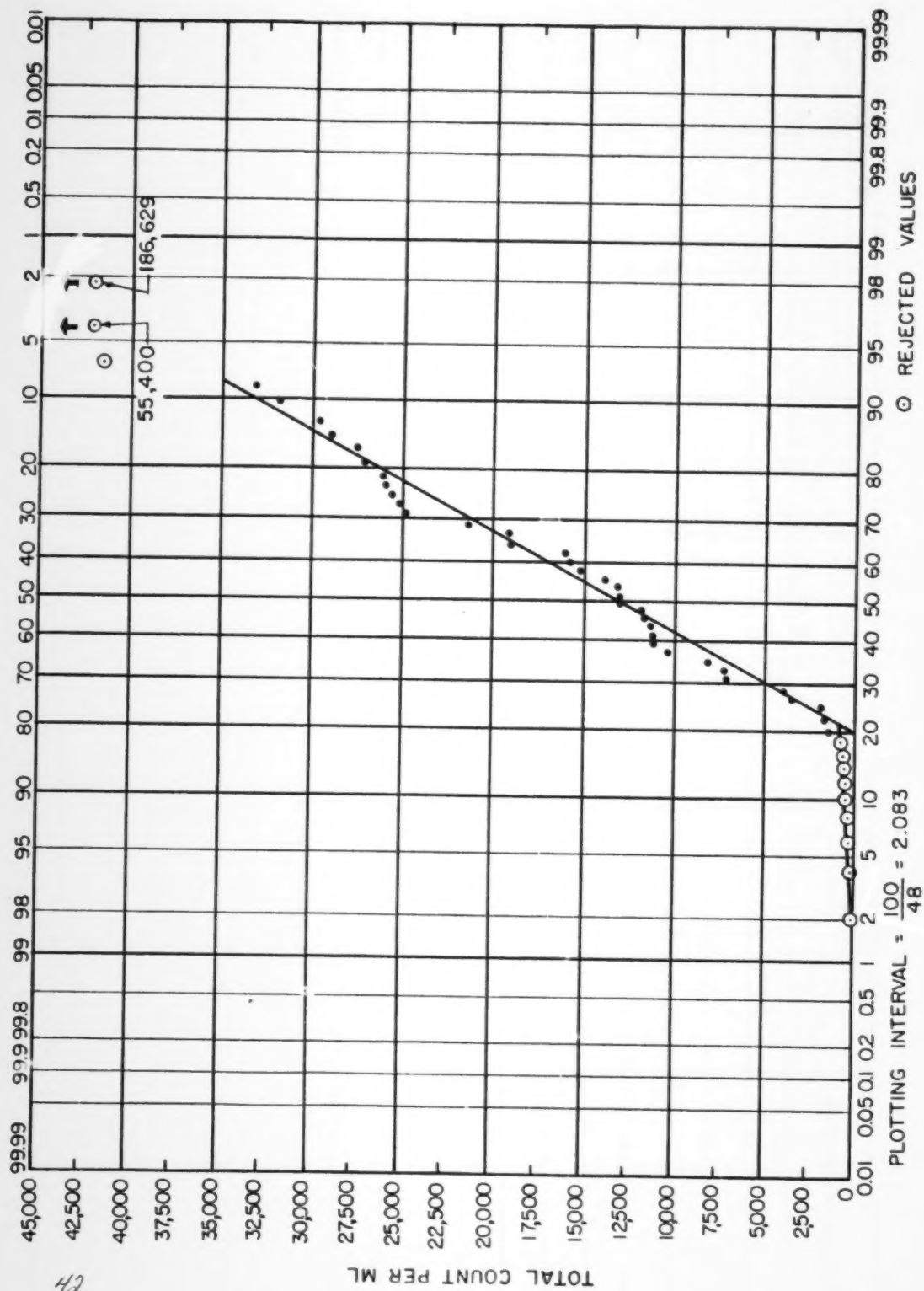
TOTAL COUNT PER ML

TOTAL COUNT PER ML			
COUNT 1	COUNT 2	COUNT 3	AVERAGE

DIFFERENTIAL COUNT

[illegible]

(Continued on second sheet)



PROBABILITY CURVE FOR PARTICIPANTS

ANALYTICAL REFERENCE SERVICE MEMBERSHIP

STATE AGENCIES

Alabama Water Improvement Commission
Arizona State Department of Health
Arkansas State Board of Health
California State Department of Public Health (Berkeley)
California State Department of Public Health (Los Angeles)
California Department of Water Resources
California Disaster Office
Colorado State Department of Public Health
Connecticut State Department of Health
Delaware Water Pollution Commission
Florida State Board of Health (Bureau of Laboratories, Jacksonville)
Florida State Board of Health (Division of Sanitary Engineering, Jacksonville)
Florida State Board of Health (Pensacola)
Florida State Board of Health (Winter Haven)
State of Hawaii Department of Health
Idaho Department of Health
Illinois State Department of Public Health (Chicago)
Illinois State Department of Public Health (Springfield)
Illinois State Water Survey Division
Indiana State Board of Health
Kansas State Board of Health (Sanitary Engineering Laboratories)
Kentucky State Department of Health
Louisiana State Board of Health
Maryland State Department of Health
Maryland State Water Pollution Control Commission
Massachusetts Department of Public Health
Massachusetts General Hospital
Michigan State Department of Health
Michigan Water Resources Commission
Minnesota State Department of Health
Missouri Department of Public Health and Welfare
Montana State Board of Health
Nebraska State Department of Health
Nevada State Department of Health (Las Vegas)
Nevada State Department of Health (Reno)
New Hampshire State Department of Health
New Hampshire Water Pollution Commission

New Jersey State Department of Health
New Mexico State Department of Public Health
New York State Department of Health
New York State Conservation Department
North Carolina Department of Water Resources
North Dakota State Department of Health
Ohio State Department of Health
Oklahoma State Department of Health
Oregon State Board of Health
Pennsylvania Department of Health
Puerto Rico Institute of Health Laboratories
Rhode Island Department of Health
South Carolina Water Pollution Control Authority
South Dakota Department of Health
Tennessee Division of Preventable Diseases
Tennessee Stream Pollution Control Board
Texas State Department of Health
Utah State Department of Health
Vermont State Department of Health
Vermont State Department of Water Resources
Virginia State Department of Health
Virginia State Water Control Board
Washington State Department of Health
West Virginia State Water Resources Commission
Wisconsin State Board of Health

MUNICIPAL AGENCIES

Air Pollution Control District, Pasadena
Baltimore City Health Department
Beaumont Health Department, Beaumont, Texas
Chicago Department of Air Pollution Control
Chicago Department of Water and Sewers, South District Filtration Plant
City of Charlotte, North Carolina, Water Department
City of Dayton, Department of Water
City of Durham, Department of Water Resources
City of Miami, Alexander Orr Jr., Water Treatment Plant
City of New York, Department of Health
City of Pasadena, Department of Public Health
Dayton Department of Service and Buildings
Erie County Laboratory (Buffalo)
Flint Water Plant, Department of Public Works and Utilities
Long Beach Water Department

Los Angeles County Flood Control District
Los Angeles Department of Public Works (Hyperion Treatment Plant)
Los Angeles Department of Water and Power
Louisville Water Company, Incorporated
Metropolitan Water District of Southern California
Omaha Metropolitan Utilities District
Philadelphia Department of Public Health
Philadelphia Department of Public Health (Occupational Environment Section)
Philadelphia Suburban Water Company
Philadelphia Water Department (Belmont Laboratory)
Philadelphia Water Department (Torresdale Laboratory)
St. Louis County Water Company
St. Louis Department of Health and Hospitals
Topeka, Kansas, Water Works

FEDERAL AGENCIES

Associated Universities, Incorporated, Brookhaven National Laboratory
DHEW, PHS, Great Lakes - Illinois River Basin Project
DHEW, PHS, Northeastern Radiological Health Laboratory, Winchester, Massachusetts
DHEW, PHS, Off-Site Radiological Safety Program, Las Vegas, Nevada
DHEW, PHS, Water Quality Section, Division of Water Supply and Pollution Control, Cincinnati, Ohio
Food and Drug Administration, Division of Pharmacology, Washington, D. C.
Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tennessee
Pearl Harbor Naval Shipyard
Sixth U.S. Army Medical Laboratory, Fort Baker, California
Tennessee Valley Authority, Chattanooga, Tennessee (Stream Pollution Control)
Tennessee Valley Authority, Wilson Dam, Alabama (Occupational Health Branch)
2793D U.S. Air Force Hospital, Regional Environmental Health Laboratory, McClelland Air Force Base, California
2794th U.S. Air Force Dispensary - Class B, Kelly AFB, Texas
U.S. Air Force Radiological Health Laboratory, Wright-Patterson AFB, Ohio
U.S. Army Environmental Hygiene Agency, Maryland
U.S. Department of the Interior, Bureau of Reclamation, Denver, Colorado
U.S. Department of the Interior, Geological Survey, Columbus, Ohio
U.S. Department of the Interior, Geological Survey, Denver, Colorado
U.S. Department of the Interior, Geological Survey, Philadelphia, Pennsylvania
U.S. Department of the Interior, Geological Survey, Sacramento, California

UNIVERSITIES

Case Institute of Technology, Department of Civil and Sanitary Engineering
Columbia University
University of Florida, Department of Chemistry
Georgia Institute of Technology, Department of Applied Biology
Johns Hopkins University, School of Hygiene and Public Health
University of Kansas, School of Engineering and Architecture
New Mexico State University, Department of Civil Engineering
University of North Carolina, Chapel Hill, North Carolina
University of Pittsburgh, Graduate School of Public Health
Purdue University, Department of Chemistry
Rensselaer Polytechnic Institute, Troy, New York
Rutgers - The State University, Department of Sanitation
Washington State University, Division of Industrial Research

INDUSTRY

American Cyanamid Company, Bound Brook, New Jersey
Anaconda Company, Grants, New Mexico
Atlantic Refining Company, Philadelphia, Pennsylvania
Battelle Memorial Institute, Columbus, Ohio
Bethlehem Steel Company, Bethlehem, Pennsylvania
California Water Service Company, San Jose, California
Central Farmers Fertilizer Company, Montpelier, Idaho
Culligan, Incorporated, Northbrook, Illinois
Dearborn Chemical Company, Chicago, Illinois
Dow Chemical Company, Midland, Michigan
E. I. du Pont de Nemours and Co., Aiken, South Carolina
Ekroth Laboratories, Inc., Brooklyn, New York
Fairbanks, Morse, and Company, Research Center, Beloit, Wisconsin
Food Machinery and Chemical Corporation, Pocatello, Idaho
General Electric Company, Louisville, Kentucky
Goodyear Atomic Corporation, Piketon, Ohio
HALL Laboratories Division, Calgon Corporation, Pittsburgh, Pennsylvania
Hazleton Nuclear Science Corporation, Palo Alto, California
Industrial Chemicals, Inc., South Bend, Indiana
Ionics, Incorporated, Cambridge, Massachusetts
Kennecott Copper Corporation, Salt Lake City, Utah
Minute Maid Company, Anaheim, California
Monsanto Chemical Company, St. Louis, Missouri

NALCO Chemical Company, Chicago, Illinois
Pacific Gas and Electric Company, Emeryville, California
Pan American World Airways, Patrick Air Force Base, Florida
Pomeroy and Associates, Pasadena, California
Radiation Detection Company, Mountain View, California
Reynolds Electrical and Engineering Company, Las Vegas, Nevada
Roy F. Weston, Inc., Newtown Square, Pennsylvania
Sandia Corporation, Sandia Base, Albuquerque, New Mexico
Tracerlab, Inc., Richmond, California
U.S. Industrial Chemicals Company, Tuscola, Illinois
Water Service Laboratories, Inc., New York, New York

FOREIGN

Beograd, Yugoslavia, University of Beograd
Bogota, Colombia, South America, National University of Colombia
Caracas, Venezuela, Instituto Nacional de Obras Sanitarias
Chesterfield, Derbyshire, England, The British Coke Research Association
Didcot, Berks, England, United Kingdom Atomic Energy Authority
Hamilton, Ontario, Canada, Department of Municipal Laboratories
Kingston, Jamaica, West Indies, Scientific Research Council
Leatherhead, Surrey, England, Central Electricity Research Laboratories
Leeds, England, Houldsworth School of Applied Science, The University of Leeds
London, England, The Permutit Company, Ltd.
Marlow, Buckinghamshire, England, The Water Research Association
Ottawa, Ontario, Canada, Department of National Health and Welfare
Ottawa, Ontario, Canada, Occupational Health Division, Department of
National Health and Welfare
Sao Paulo - Brasil, Comissao Inter-Municipal de Controle da Poluicao das
Agua e Do Ar
Sydney, Australia, Metropolitan Water, Sewerage, and Drainage Board
Taiwan Institute of Environmental Sanitation, PHA, Taiwan
Pingtung Air Pollution Laboratory
Pingtung Organic Waste Laboratory
Taichung Water Laboratory
Tainun Water Laboratory
Taipei Milk Laboratory
Taipei Radiological Health Laboratory
Taipei Water Laboratory
Taitung Water Laboratory
Vancouver, B. C., Canada, Department of Health Services and Hospital Insurance
Vancouver, B. C., Canada, Department of National Health and Welfare

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